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Oncogenic kinase signalling

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Protein-tyrosine kinases (PTKs) are important regulators of intracellular signal-transduction pathways mediating development and multicellular communication in metazoans. Their activity is normally tightly controlled and regulated. Perturbation of PTK signalling by mutations and other genetic alterations results in deregulated kinase activity and malignant transformation. The lipid kinase phosphoinositide 3-OH kinase (PI(3)K) and some of its downstream targets, such as the protein-serine/threonine kinases Akt and p70 S6 kinase ($p70^{S6K}$), are crucial effectors in oncogenic PTK signalling. This review emphasizes how oncogenic conversion of protein kinases results from perturbation of the normal autoinhibitory constraints on kinase activity and provides an update on our knowledge about the role of deregulated PI(3)K/Akt and mammalian target of rapamycin/$p70^{S6K}$ signalling in human malignancies.

Deregulated (that is, autonomous) cell growth is the defining feature of all neoplasms, both benign and malignant. Malignant neoplasms have, in addition, the capacity to invade normal tissues and metastasize to and grow at distant body sites, the other main defining criterion of cancer. Deregulated cell growth occurs as a result of perturbed signal transduction defined, in its broadest sense, as all cellular signals that modulate or alter cellular behaviour or function[1,2]. Consequently, cancers do not necessarily arise as a result of an increased rate of cell proliferation. Rather, it is the critical balance between the rate of cell-cycle progression (cell division) and cell growth (cell mass) on one hand, and programmed cell death (apoptosis) on the other, that is important[3]. During normal embryonic development and in adult life, signalling needs to be precisely coordinated and integrated at all times, and properly regulated differentiation signals are critical for preventing oncogenesis. The old dogma stating an inverse relation between cell-differentiation stage and (deregulated) cell proliferation (a malignant tumour tends to be more de-differentiated than its parent cell type) illustrates this important principle.

Certain classes of signalling proteins and pathways are targeted much more frequently by oncogenic mutations than others[4,5]. Thus, molecules governing extracellular growth, differentiation and developmental signals, in particular, are often mutated in cancers. One illustrative example is provided by receptor protein-tyrosine kinases (RPTKs), a subclass of transmembrane-spanning receptors endowed with intrinsic, ligand-stimulatable PTK activity. When mutated or altered structurally, RPTKs can become potent oncoproteins, causing cellular transformation. Conversely, RPTK activity in resting, untransformed cells is normally tightly controlled. Recent studies have provided new insights regarding the structural bases for normal intramolecular control of PTKs, and shown that multiple 'layers' of autoinhibitory mechanisms operate as a safeguard against unwanted protein kinase activation. Hence, rather than looking at oncogenic mechanisms as 'activating events' or 'hits', whether due to mutations, overexpression or structural re-arrangements, it is more meaningful to see them as mechanisms causing primarily relief or obstruction of normal autoinhibitory and regulatory constraints. This review presents some examples of perturbed PTK signalling mechanisms, as well as providing an update on recent insights regarding the role of PI(3)K and ribosomal $p70^{S6K}$ in oncogenesis.

RPTK regulation by autoinhibition

The sequencing effort of the Human Genome Project has revealed that up to ~20% of the ~32,000 human coding genes encode proteins involved in signal transduction, including transmembrane receptors, G-protein subunits and signal-generating enzymes. Among these, are more than 520 protein kinases and 130 protein phosphatases, exerting tight and reversible control on protein phosphorylation. Both of these enzyme categories can be subdivided into tyrosine- or serine/threonine-specific, based on their catalytic specificity. In addition, some possess dual specificity for both tyrosine and serine/threonine, and a few members of the phosphatidylinositol kinase family also exhibit protein-serine/threonine kinase activity. At the time of writing there are >90 known PTK genes in the human genome; 58 encode transmembrane RPTKs distributed into 20 subfamilies, and 32 encode cytoplasmic, non-receptor PTKs in 10 subfamilies[6,7] (Figs 1, 2). Of the ~30 tumour-suppressor genes and >100 dominant oncogenes known to date[8], protein kinases, in particular PTKs, comprise a large fraction of the latter group (Table 1). PTKs are also the largest group of dominant oncogenes with structural homology. PTKs evolved to mediate aspects of multicellular communication and development; they are found only in metazoans, where they comprise ~0.3% of genes. Somatic mutations in this very small group of genes cause a significant fraction of human cancers, again emphasizing the inverse relationship between normal developmental regulation and oncogenesis.

The physiological regulation of RPTKs is key to understanding the mechanisms causing their oncogenic activation (see refs. 9,10 for recent reviews). Signalling by RPTKs requires ligand-induced receptor oligomerization, which results in tyrosine autophosphorylation of the receptor subunits[11]. This both activates catalytic activity and generates phosphorylated tyrosine residues that mediate the specific binding of cytoplasmic signalling proteins containing Src homology-2 (SH2) and protein tyrosine-binding (PTB) domains. Crystal structures of the inactive forms of the core cytoplasmic kinase domains of the insulin receptor (InsR), fibroblast growth factor receptor-1

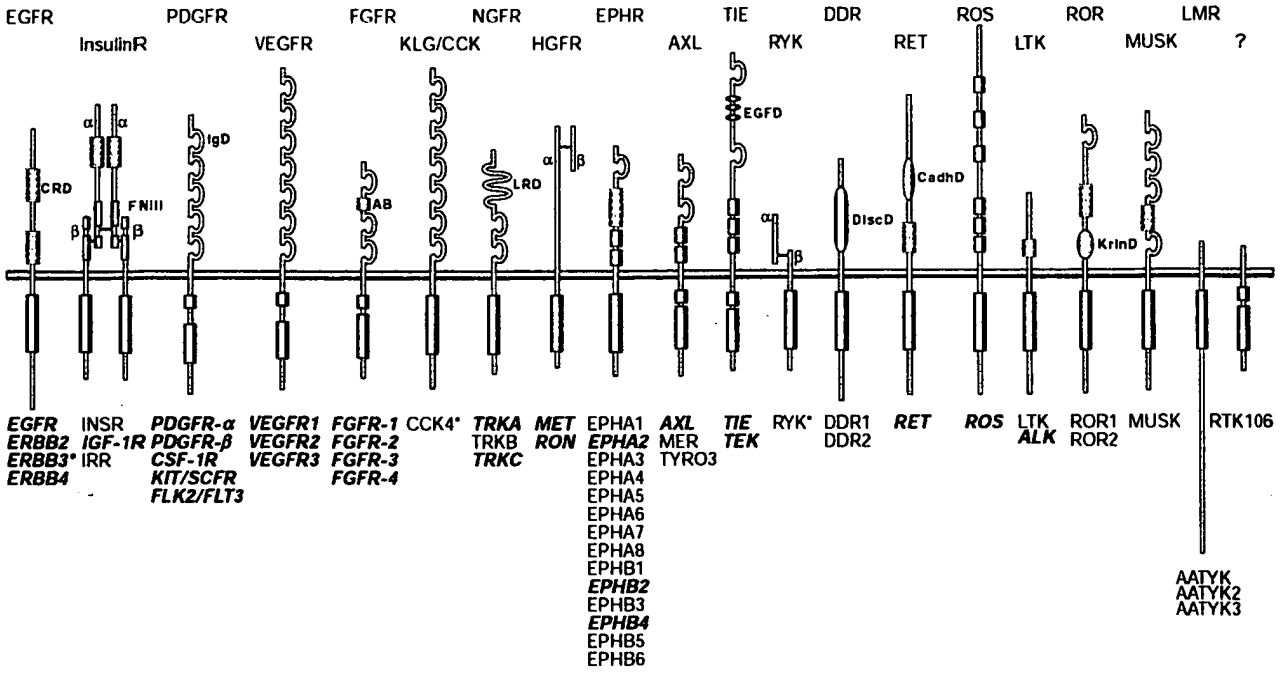

Figure 1 Human receptor protein-tyrosine kinases. The prototypic receptor for each family is indicated above the receptor, and the known members are listed below. Abbreviations of the prototypic receptors: EGFR, epidermal growth factor receptor; InsR, insulin receptor; PDGFR, platelet-derived growth factor receptor; VEGFR; vascular endothelial growth factor receptor; FGFR, fibroblast growth factor receptor; KLG/CCK, colon carcinoma kinase; NGFR, nerve growth factor receptor; HGFR, hepatocyte growth factor receptor, EphR, ephrin receptor; Axl, a Tyro3 PTK; TIE, tyrosine kinase receptor in endothelial cells; RYK, receptor related to tyrosine kinases; DDR, discoidin domain receptor; Ret, rearranged during transfection; ROS, RPTK expressed in some epithelial cell types; LTK, leukocyte tyrosine kinase; ROR, receptor orphan; MuSK, muscle-specific kinase; LMR, Lemur. Other abbreviations: AB, acidic box; CadhD, cadherin-like domain; CRD, cysteine-rich domain; DiscD, discoidin-like domain; EGFD, epidermal growth factor-like domain; FNIII, fibronectin type III-like domain; IgD, immunoglobulin-like domain; KrinD, kringle-like domain; LRD, leucine-rich domain. The symbols α and β denote distinct RPTK subunits. RPTK members in bold and italic type are implicated in human malignancies (see Table 1). An asterisk indicates that the member is devoid of intrinsic kinase activity.

(FGFR1) and Flk1 (vascular endothelial growth factor receptor-2 or VEGFR2) tyrosine kinases have provided a molecular understanding of the tight control of catalytic activity resulting from a *cis* inhibition/*trans* activation mechanism[12].

In both the unstimulated FGFR1 and VEGFR2, the activation loop occludes substrate tyrosine binding to the active site, whereas in the unstimulated InsR, Tyr1162 in the activation loop is bound in *cis* in the active site to prevent substrate access, and the beginning of the activation loop occludes ATP binding. In response to ligand stimulation of RPTKs, one or several of the tyrosine residues in the activation loop is phosphorylated in *trans* by the dimeric receptor partner. This leads to repositioning of the activation loop away from the active site, allowing substrate (and in the case of the InsR, ATP) access. An equilibrium exists between inactive and active loop conformations of the unphosphorylated RPTKs in solution[13]. This equilibrium enables *cis* inhibition, yet allows phosphorylation in *trans* between ligand-induced receptor dimers. The importance of this is supported by several examples of oncogenic point mutations in the activation loop that cause constitutive RPTK activation. A simplified diagram of RPTK activation is presented in Fig. 3a.

Evidence indicates that RPTK dimerization per se is not always sufficient for kinase activation. There seems to be an additional requirement for ligand-induced conformational switches, ensuring that the catalytic domains are juxtaposed in a proper configuration to enable phosphorylation in *trans* between receptor subunits[9,14]. Accordingly, other regions of RPTKs can have autoinhibitory functions in unstimulated cells. For instance, in Tek (TIE2), the carboxy-terminal tail partially occludes the substrate tyrosine-binding site[15]. Autophosphorylation of the tail region exposes its phosphotyrosine residues for substrate binding, as well as the substrate-binding site in the kinase. In addition, for some RPTKs, including platelet-derived growth factor receptor (PDGFR), Kit/stem-cell factor receptor (SCFR), colony-stimulating factor-1 receptor (CSF1-R), ephrin receptor (EphR) and InsR, the juxtamembrane region has been implicated in autoinhibition. Hence, autophosphorylation of one or two homologous juxtamembrane tyrosine residues in several of these receptors is required for full kinase activation, and mutation to phenylalanine significantly reduces kinase activation (see ref. 16 and references therein).

Autophosphorylation of these residues seems to serve a dual function: it relieves the inhibitory conformation enabling full kinase activation and at the same time creates binding sites for numerous SH2-containing signalling molecules, such as Src, RasGAP, SHP-1, SHEP-1 and PI(3)K. Consistent with a repressive function of the juxtamembrane region, substitution of a Val residue just amino-terminal to the regulatory tyrosine residues in the PDGFR-β results in constitutive kinase activation[17]. In addition, numerous oncogenic mutations in human Kit are located either N- or C-terminal to the two tyrosines, and internal gene duplications in the juxtamembrane region of Flk2/Flt3 result in constitutive kinase activity[18,19]. The crystal structure of the EphB2 kinase domain has revealed a possible mechanism for the inhibition by its juxtamembrane region. The unphosphorylated juxtamembrane region impinges on the Cα helix in the N-terminal kinase lobe and other regions of the kinase, resulting in catalytic repression. The structure also suggests how phosphorylation relieves repression by causing dissociation of the juxtamembrane region (L. Groot, B. Baskin, T. Pawson & F. Sicheri, personal communication).

Deregulation of RPTK by relief of restraints

How do these insights apply to cellular transformation and cancer? In principle, for all PTKs involved in cancer, oncogenic deregulation results from relief or perturbation of one or several of the

auto-control mechanisms that ensure the normal repression of catalytic domains. A little more than half (31) of the known RPTKs have been repeatedly found in either mutated or overexpressed forms associated with (human) malignancies, including sporadic cases (see Table 1).

There are four main principles for oncogenic transformation by PTKs. First, retroviral transduction of a proto-oncogene corresponding to a PTK concomitant with deregulating structural changes is a common transforming mechanism in rodents and chicken. Second, genomic re-arrangements, such as chromosomal translocations, can result in oncogenic fusion proteins that include (minimally) a PTK catalytic domain and an unrelated protein that provides a dimerization function. Third, gain-of-function (GOF) mutations or small deletions in RPTKs and cytoplasmic PTKs are associated with several malignancies. Finally, PTK overexpression resulting from gene amplification is associated with several common human cancers. In general, the transforming effect can be ascribed to enhanced or constitutive kinase activity with quantitatively or qualitatively altered downstream signalling.

RPTK overexpression leads to constitutive kinase activation by increasing the concentration of dimers. Important examples are the Neu/ErbB2 and epidermal growth factor receptor (EGFR), which are often amplified in breast and lung carcinomas (Table 1). ErbB2 signalling is inhibited by binding of the monoclonal antibody herceptin, which is being used in the treatment of ErbB2-positive breast cancers. A selective small-molecule EGFR-tyrosine kinase inhibitor, ZD1839 ('Iressa'), is in late phase trials for advanced non-small-cell lung cancer. Cancers due to chimaeric RPTK domains also involve constitutive kinase activation, which depends on the oligomerization domain(s) of the N-terminal fusion partner. Enforced dimer formation juxtaposes the catalytic domains in an optimal orientation for *trans* phosphorylation, probably very similar to a ligand-induced receptor dimer.

Among the more than 30 RPTKs implicated in human cancer, some, such as ErbB2 and EGFR that are amplified, mutated and/or overexpressed in prevalent cancers (Table 1), have been extensively reviewed (see, for example, ref. 20). Here, we will restrict our discussion to the Ret/glial-derived neurotrophic factor receptor (GDNFR) and Kit/SCFR RPTKs, which illustrate a variety of mechanisms for RPTK-induced oncogenic transformation.

Ret/GDNFR

Ret is required for development of the kidneys and enteric system and for neuronal differentiation and survival. It is part of a multicomponent receptor for the glial-derived neurotrophic factor (GDNF) family of neurotrophins, which include neurturin (NTN), artemin (ART) and persephin (PSP). In response to ligand, Ret is activated by heterodimer formation with one of four structurally related glycosylphosphatidylinositol (GPI)-linked cell-surface receptors, GFR-α1–4. At least eight common somatic rearrangements result in fusions between the N terminus of various proteins and the PTK domain of Ret (see Table 1 and ref. 21). This leads to subsequent GDNF- and NTN-independent kinase activation caused by constitutive dimerization of the fusion proteins, resulting in papillary thyroid carcinomas (PTCs). Somatic Ret GOF mutations are found in some sporadic tumours, while germline Ret GOF mutations are involved in three familial tumour syndromes — multiple endocrine neoplasia 2A (MEN2A), MEN2B and familial medullary thyroid carcinoma (FMTC; see Table 1).

All the identified oncogenic mutations cluster in extracellular-domain exons 10, 11 and 13–16 of the PTK domain. Almost 100% of patients with MEN2A and FMTC have mutations in one of the five conserved cysteines in the extracellular domain of Ret, causing formation of intermolecular disulphide bonds between Ret molecules, and constitutive dimerization and activation[22]. In contrast, MEN2B is due to a recurring Met918Thr mutation (a methionine-to-threonine substitution at codon 918), which activates by a different mechanism. Met918 corresponds to a highly conserved Met just upstream of the Ala-Pro-Glu motif (PTK subdomain VIII) in the substrate-binding pocket of RPTKs, whereas a threonine residue at this position is typical for cytoplasmic PTKs, like c-Src. This replacement increases the kinase activity of Ret without constitutive dimer formation[22], most likely because it enables substrate access without prior autophosphorylation of the activation loop. In addition, it alters the substrate specificity of the Ret/MEN2B receptor towards peptide substrates that are optimal for Src and Abl[23,24]. The altered specificity leads to autophosphorylation of Ret on novel tyrosine

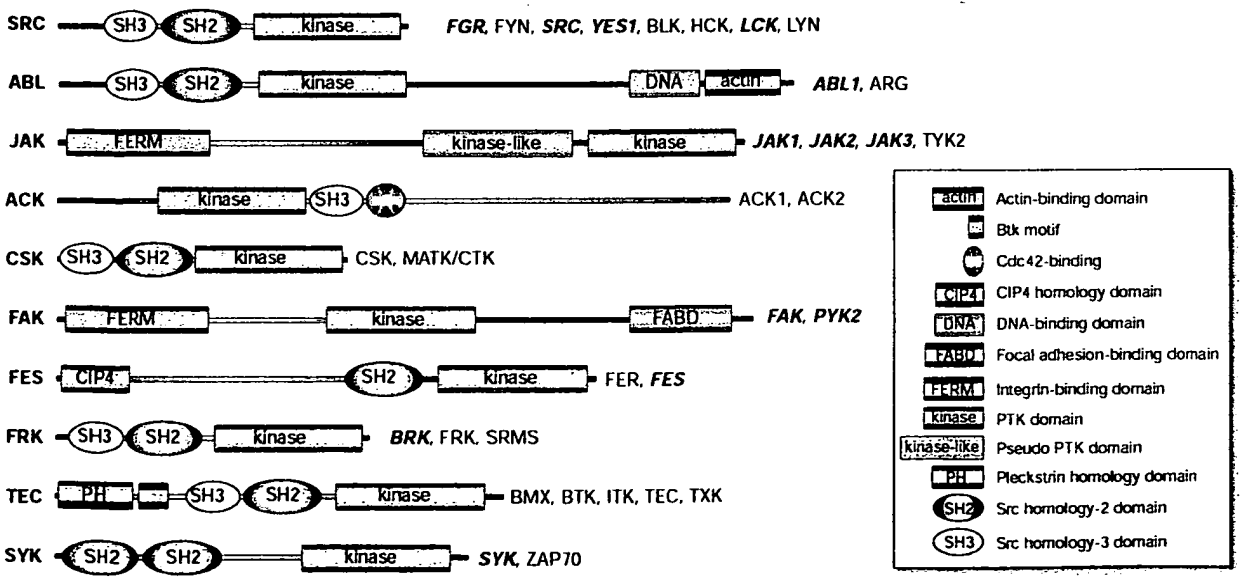

Figure 2 Human cytoplasmic protein-tyrosine kinases. The family members are indicated to the right and the family name to the left of each PTK. The PTK members in bold and italic type are implicated in human malignancies (see Table 1).

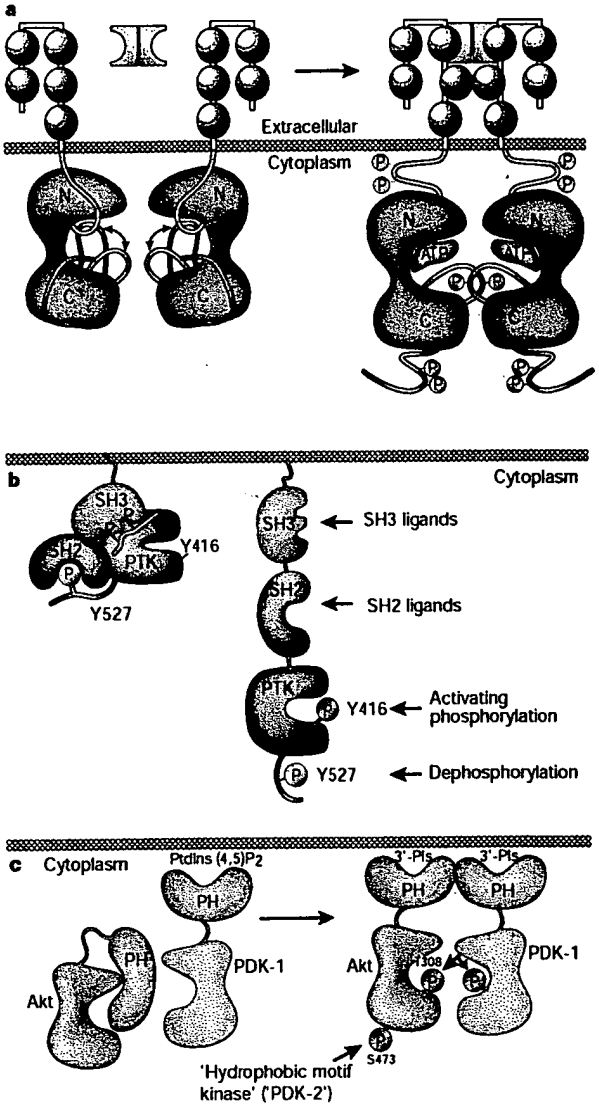

Figure 3 Protein kinase activation mechanisms. **a**, RPTK activation. Left: RPTK kinase activity is tightly repressed in the unstimulated state. The activation and catalytic loops exist in an equilibrium between a substrate-precluding (blue) and substrate-accessible (green) conformation. In addition, the juxtamembrane region (orange) and C-terminal region (red) might interfere with the conformation of the N-terminal kinase lobe ('N') and/or substrate access. Right: ligand-induced receptor dimerization and tyrosine autophosphorylation result in relief of the inhibitory constraints exerted by the activation loop, and the juxtamembrane and C-terminal regions. **b**, c-Src activation. Left: c-Src kinase activity is tightly repressed in the unstimulated state. The SH2 domain interacts with phospho-Tyr 527 in the C terminus and the SH3 domain with the polyproline type II helix in the linker region between the SH2 and kinase domain. This causes misalignment of residues that are critical for kinase activity. Right: binding of ligands to the SH2 or SH3 domain and/or dephosphorylation of phospho-Tyr 527 by PTPs relieves the inhibitory constraints on the kinase. **c**, Akt activation. Left: it is thought that the N-terminal PH domain precludes kinase access to and phosphorylation of the activation-loop Thr 308 by PDK-1. Right: PI(3)K activation results in production of PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2, which recruits Akt to the membrane by binding to its PH domain. This exposes Thr 308 for phosphorylation by PDK-1, which is already located at the membrane. An unidentified PDK-2 kinase phosphorylates Ser 473 in the C terminus, which leads to full Akt activation. See text for details.

residues and tyrosine phosphorylation of substrates that are not phosphorylated by the activated wild-type Ret. However, there is also increased activation of PI(3)K, which might be crucial for transformation[25].

A recently developed *in vivo* model for MEN2B, based on introduction of the Met918Thr homologous mutation into the germline of mice, will enable studies of different signal-transduction pathways from Ret/MEN2B involved in tumorigenesis[26]. Besides the Met918Thr mutation, a number of other recurrent point mutations have been found in Ret in MEN2A, MEN2B and FMTC. In all cases, the activating mutations occur in highly conserved regions of the Ret PTK domain that are normally involved in kinase repression in the inactive receptor. Different tyrosine residues are likely to be autophosphorylated in the different Ret mutants, resulting in binding and activation of different signalling molecules[27]. Based on mutagenesis studies, Grb2 and Shc acting upstream of the classical mitogenic Ras-Raf-ERK (for extracellular signal-regulated protein kinase) cascade and PI(3)K seem important for the transforming effects.

Kit/SCFR

The Kit/SCFR provided the first example of naturally occurring, germline loss-of-function (LOF) point mutations in a mammalian RPTK, and the resulting phenotypes established the importance of this receptor for normal haematopoiesis and mast-cell development, melanogenesis, gametogenesis and development of interstitial cells of Cajal[28,29]. Recently, more than 30 GOF mutations, either single amino-acid changes or deletions of a few amino acids, have been identified in the Kit/SCFR, and they are associated with several highly malignant tumours in humans (reviewed in ref. 30). The mutations tend to cluster in two regions. Those in exon 11 contained in the juxtamembrane region are associated with gastrointestinal stromal tumours, whereas recurrent exon 17-mutations of Asp816 to either Val or His in the second half of the kinase domain are associated with mast-cell/myeloid leukaemias and seminomas/dysgerminomas, respectively.

The transforming mechanism for both of the main types of mutation involves dimer formation resulting in constitutive ligand-independent kinase activation. In most patients, tumours are heterozygous for the mutant form of c-*kit*, which indicates a dominant-positive phenotype. This is consistent with constitutively active heterodimers formed between the mutant and wild-type Kit receptors. The mutations in the juxtamembrane region cluster around the two autophosphorylation sites, Tyr 568 and 570, involved in binding c-Src and SHP-1 (an SH2 domain-containing protein-tyrosine phosphatase (PTP) expressed in haematopoietic cells)[31,32], and the presence of mutant Kit implies a worse prognosis[33]. The

juxtamembrane mutations probably relieve the repressive effect of this domain on PTK activity, allowing limited autophosphorylation of the kinase domain, which results in additional activating conformational changes enabling full kinase activation[13]. It is of note that the frequently mutated Asp816 in Kit is a highly conserved residue just C-terminal to the conserved Asp-Phe-Gly motif in the activation loop of protein kinases. Mutation of the corresponding residue in Met and Ret results in papillary renal and thyroid carcinomas, respectively[34]. This mutation seems to shift the equilibrium of the activation loop in unstimulated RPTKs towards the active conformation[13].

Among the many Kit-induced signalling pathways, PI(3)K is particularly important and seems crucial for oncogenic transformation by GOF point-mutant Kit receptors[35,36]. SHP-1 might also be an important target for inactivation in Kit-induced oncogenesis. SHP-1 binds to the juxtamembrane tyrosine autophosphorylation sites in Kit and CSF-1R[31,37], resulting in phosphatase activation and direct receptor dephosphorylation. The importance of SHP-1 in negative regulation of Kit and CSF-1R signalling is supported by the phenotype of *Motheaten* (*Me*)-mutant mice, which have LOF mutations of SHP-1, and the partial phenotypic rescue obtained by crossing them to *Dominant white-spotting W*-mutant mice, which have naturally occurring LOF mutations in Kit[38]. Asp816Val-Kit causes enhanced degradation of SHP-1 through the proteasome pathway[39], and alternative transcripts causing LOF mutations or truncations of SHP-1 are frequent in primary Kit-expressing tumours from leukaemic patients[40]. Thus, SHP-1 might be a tumour suppressor for Kit-induced malignancies. Clinical trials with the small-molecule Bcr-Abl PTK inhibitor STI571 (ref. 41), which also inhibits Kit/SCFR and PDGFR-β[42], have been initiated for Kit-positive gastrointestinal stromal tumours. The small-molecule PTK inhibitors SU5416 and SU6668, originally developed for VEGFR as angiogenesis inhibitors, also inhibit Kit and are entering early trials this year for Kit-positive acute myeloid leukaemias.

Besides regulation by PTPs, other mechanisms for inhibition of RPTK signalling include ligand-induced receptor endocytosis, regulation of negative feedback loops, and heterodimerization with kinase-inactive RPTKs. Perturbation of these inhibitory mechanisms might result in RPTK-induced malignancies or sensitize cells for oncogenic transformation under some circumstances.

Cytoplasmic protein-tyrosine kinases

Given their importance in receptor signalling pathways, it might come as a surprise that of the 32 known cytoplasmic PTKs (Fig. 2), less than half have been implicated convincingly in human cancer (Table 1). The bias comes from the fact that most of the initial studies were on the viral counterparts, and aimed at elucidating their transforming mechanisms.

c-Src

c-Src was the first cellular homologue of a viral oncoprotein to be discovered[43]; it is important for mitogenic signalling from many RPTKs, and has been implicated in a variety of cancers (reviewed in ref. 44). Just as for RPTKs, there is normally tight control of c-Src kinase activity through intramolecular interactions. In inactive c-Src, a C-terminal tyrosine residue (527 in mouse, 530 in human c-Src), lacking in deregulated v-Src, is phosphorylated and interacts with the SH2 domain, while the c-Src SH3 domain interacts with the linker region between the SH2 domain and the N-terminal kinase lobe. The SH2 and SH3 intramolecular interactions repress kinase activity by displacing the Cα helix in the N-terminal lobe and by positioning the activation loop to block access to the active site. Accordingly, c-Src can be de-repressed not only upon dephosphorylation of phosphorylated Tyr527, but also by binding through its SH2 domain to specific tyrosine autophosphorylation sites in ligand-stimulated RPTKs, resulting in SH2 displacement from phosphorylated Tyr527, or by binding of the SH3 domain to Pro-X-X-Pro motifs in target proteins[45]. This results in autophosphorylation in *trans* of the conserved activation loop Tyr416 and stabilization of the active conformation. This activation mechanism is similar to that of RPTKs, in that the activating event (ligand binding and dimerization for RPTKs, and SH2- or SH3-domain ligand engagement for c-Src) results in removal of inhibitory constraints on the kinase domain (Fig. 3a, b).

Several PTPs are implicated in regulating c-Src through dephosphorylation of the C-terminal c-Src kinase (CSK) phosphorylation site, Tyr527, including receptor-like PTPα, PTPλ and RPTPε, and the cytoplasmic PTP1B, SHP-1 and SHP-2. Elevated activity or expression of several of these PTPs correlate with enhanced levels of c-Src kinase activity in a number of transformed cells[44]. But the most direct demonstration that c-Src is involved in human cancer was the identification of a mutant c-Src with truncation of the C terminus, ending with Tyr530 in human colon cancer[46]. This mutant has deregulated kinase activity because the lack of residues C-terminal to the phosphorylated Tyr530 prevents SH2 association and establishment of the inactive conformation. Again, oncogenic perturbation results from relief of the tightly controlled constraints on kinase activity. It is not clear which signalling pathways are important for c-Src transformation, but a dominant-negative mutant of signal transducer and activator of transcription (STAT)-3 blocks v-Src transformation and c-Myc induction, indicating that STATs might be involved.

c-Abl

The Philadelphia (Ph) chromosome provided the first example of a consistent chromosomal abnormality associated with a specific type of leukaemia[47]. The t(9; 22) reciprocal translocation involves the non-receptor PTK c-Abl on chromosome 9 and a breakpoint cluster region on chromosome 22. A majority of patients with chronic myeloid leukaemia, and a significant fraction of Ph-positive patients with acute lymphocytic leukaemia have one of three different versions of this translocation (Table 1). The c-Abl protein is structurally complex, consisting of SH3, SH2, PTK, DNA-binding and actin-binding domains (Fig. 2), among others.

Recent evidence suggests that nuclear c-Abl has a role primarily in DNA damage-induced apoptosis, and that Bcr-Abl circumvents this function because it is retained in the cytoplasm[48]. Hence, nuclear c-Abl is activated by ionizing radiation and certain cytostatic drugs, and the activation is dependent on ataxia telangiectasia-mutated (ATM), a protein-serine/threonine kinase belonging to a family of proteins possessing a phosphoinositide kinase-homology domain. Conversely, the tumour suppressor Rb, which binds to c-Abl in the G0 and G1 phases of the cell cycle and represses the tyrosine kinase function of c-Abl, prevents the DNA damage-induced activation of nuclear c-Abl, which is seen only after entry into S-phase, when c-Abl is released by Rb hyperphosphorylation. Consistently, Rb-deficient cells are more sensitive to DNA damage-induced cell death (reviewed in ref. 49).

The possible mediators of c-Abl-induced cell death include the transcription factors p53 and p73, and the stress-activated mitogen-activated protein kinase (MAPK) family members c-Jun N-terminal kinase (JNK/SAPK) and p38, but p73 seems to be the crucial target. Hence, p73 accumulates in both wild-type and p53-deficient cells in response to DNA damage in a c-Abl-dependent manner, and ectopic expression of p73 induces growth arrest and apoptosis, in part by inducing p53 target genes[49]. The transcription factor E2F1, deregulated in many cancers and known to stabilize p53, directly transactivates p73, causing transcription of p53 target genes in a p53-independent manner, and apoptosis[50]. E2F1 is released from Rb during G1 exit, and so the induction of p73 can occur only in early S phase. It will be interesting to see whether c-Abl-induced apoptosis via p73 is dependent on released E2F1, which would explain why Abl induces apoptosis only after Rb hyperphosphorylation in early S phase. The c-Abl protein also functions in the cytoplasm, where it is involved in PDGF-induced motility responses and cell adhesion[51].

Bcr-Abl is localized exclusively in the cytoplasm of transformed cells by retention mechanisms that involve Abl kinase activity and Bcr

Table 1 Examples of dominant protein-tyrosine kinase oncogenes

PTK (proto-oncogene)	Viral oncogene* (viral oncoprotein)	Oncogenic alteration	Tumour/cancer types (only the most frequent, mainly human types are described)
EGFR/ErbB1 (c-*erbB*)	v-*erbB* from AEV ($p68/74^{erbB}$)	v-*erbB*: Truncated EGFR PTK c-*erbB*: Overexpression (amplification) Extracellular domain deletions	v-ErbB: fibrosarcomas c-ErbB: mammary carcinoma, glioblastoma multiforme, ovarian, non-small-cell lung and other cancers
ErbB2/HER2/Neu		Overexpression (amplification) No recurrent human mutations (Val664Glu in rodents)	Mammary, ovarian, gastric, non-small-cell lung and colon cancer
ErbB3/HER3		Overexpression; constitutive tyrosine phosphorylation (heterodimer with ErbB2)	Mammary carcinoma
ErbB4/HER4		Overexpression	Mammary carcinoma, granulosa cell tumours
IGF-1R		Overexpression (expression required for *in vitro* transformation by many oncogenes and DNA viruses)	Cervical and other carcinomas, sarcomas
PDGFR-α		Overexpression (amplification)	Glioma, glioblastoma, ovarian carcinoma
PDGFR-β		Tel-PDGR-β (t(5; 12) translocation fusing Ets-like Tel with PDGFR-β PTK domain) Overexpression	Tel-PDGFR-β: chronic myelomonocytic leukaemia PDGFR-β: glioma
CSF-1R (c-*fms*)	v-*fms* from FeSV ($p170^{gag\text{-}fms}$)	*v-fms*: Truncated CSF-1R PTK with mutant C-terminal tail Constitutively active *c-fms*: GOF point mutations Overexpression	*v-fms*: feline sarcomas *c-fms*: acute and chronic myelomonocytic leukaemias, monocytic tumours, malignant histiocytosis, endometrial cancer, glioma
Kit/SCFR (c-*kit*)	v-*kit* from FeSV ($p80^{gag\text{-}kit}$)	v-*kit*: Truncated Kit/SCFR PTK with mutant C-terminal tail Constitutively active c-*kit*: GOF point mutations and small deletions Overexpression	v-*kit*: feline fibrosarcomas c-*kit*: malignant gastrointestinal stromal tumours, acute myeloid leukaemias, myelodysplastic syndromes, mast-cell leukaemia/systemic mastocytosis, seminomas/dysgerminomas, small-cell lung cancer and other carcinomas
Flk2/Flt3		Overexpression Internal tandem gene duplications in JM region	Haematopoietic malignancies
Flt1/VEGFR1		Expression	Tumour angiogenesis
Flk1/VEGFR2		Expression	Tumour angiogenesis
Flt4/VEGFR3		Overexpression	Tumour angiogenesis; vascular tumours (Kaposi's sarcoma, haemangiosarcoma, lymphangiosarcomas)
FGFR1		ZNF198-FGFR1 (t(8; 13) translocation fusing a novel Zn finger protein with the FGFR1 PTK domain) Overexpression Point mutations	ZNF198-FGFR1: acute myelogenous leukaemia (8p11 myeloproliferative syndrome), lymphomas Overexpression: various tumours Point mutations: autosomal skeletal disorders/dysplasias
FGFR2/K-SAM		Overexpression (amplification) and C-terminal truncation	Gastric carcinoma (mammary, prostate carcinomas)
FGFR3		IgH locus/MMSET translocation (t(4; 14) translocation placing FGFR3 PTK downstream of IgH/MMSET). Additional activating FGFR3 point mutations in skeletal dysplasias	Multiple myelomas (achondroplasia, thanatophoric dysplasia and hypochondroplasia)
FGFR4		Overexpression (amplification)	Mammary, ovarian carcinomas
TrkA		Tropomyosin(Tpm)-TrkA (t(1; 1) with N-terminal Tpm sequence fused to TrkA PTK) Tpr-TrkA (t(1; 1) with N-terminal Tpr sequence fused to TrkA PTK) Tfg-TrkA (t(1; 3) with N-terminal Tfg sequence fused to TrkA PTK domain)	Papillary thyroid carcinomas, neuroblastomas
TrkC		Tel-TrkC (t(12; 15) with H-L-H domain of Tel fused to TrkC PTK domain)	Congenital fibrosarcoma, acute myeloid leukaemias
HGFR (c-*met*)		Tpr-Met (t(1; 7) with N-terminal of Tpr fused to Met PTK domain) Overexpression GOF point mutations	Tpr-Met: Papillary thyroid carcinomas Overexpression: rhabdomyosarcoma, hepatocellular carcinoma GOF point mutations: renal carcinoma
RON		Overexpression/increased kinase activity of splice variants	Colon cancer, hepatocellular cancer
EphA2		Overexpression	Metastasizing malignant melanomas
EphB2		Overexpression	Gastric, oesophageal and colon carcinomas
EphB4		Overexpression	Infiltrating ductal mammary carcinomas
Axl		Overexpression	Acute myeloid leukaemias
TIE/TIE1		Overexpression	Capillary haemangioblastomas, haemangiopericytomas, gastric adenocarcinoma
Tek/TIE2		Expression	Tumour angiogenesis (endothelium)
Ret		Fusions: H4-Ret (PTC1), inversion; Rlα-Ret (PTC2), t(10; 17); ELE1-Ret (PTC3 & PTC4), inversion; RFG5-Ret (PTC5), inversion; HTIF1-Ret (PTC6), t(7; 10); RFG7-Ret (PTC7), t(1; 10); KTN1-Ret (PTC8), t(10; 14); ELKS-Ret, t(10; 12) GOF point mutations: primarily in MEN2A, MEN2B and FMTC (familial), but also in a few sporadic cases	PTCs and ELKS-RET: papillary thyroid carcinomas (5–30% of spontaneous carcinomas, 60–70% of radiation-induced carcinomas (Chernobyl accident) MEN2A: medullary thyroid carcinoma, parathyroid hyperplasia, pheochromocytoma MEN2B: medullary thyroid carcinoma, pheochromocytoma and enteric mucosal ganglioneuromas. FMTC: medullary thyroid carcinoma

Table 1 Examples of dominant protein-tyrosine kinase oncogenes (continued)

PTK (proto-oncogene)	Viral oncogene* (viral oncoprotein)	Oncogenic alteration	Tumour/cancer types (only the most frequent, mainly human types are described)
ROS (c-*ros*)	v-*ros* from avian UR2 SV ($p68^{gag\text{-}ros}$)	v-*ros*: Truncated Ros PTK domain. Constitutively active c-*ros*: Overexpression Rare truncations/point mutations?	v-*ros*: avian fibrosarcomas c-*ros*: glioblastomas, astrocytomas
Alk		NPM-Alk (t(2; 5) nucleophosmin fused to Alk PTK domain) Igλ-Alk (t(2; 22) Igλ fused to Alk PTK domain) Other sporadic fusions with tropomyosin, etc.	Non-Hodgkin lymphomas, CD30$^+$ and CD30$^-$ anaplastic large-cell lymphoma
Src (c-*src*)	v-*src* from RSV ($pp60^{v\text{-}src}$)	v-*src*: C-terminal truncation and point mutations (increased kinase activity) c-*src*: C-terminal truncation (increased kinase activity) Overexpression and/or increased kinase activity	$pp60^{v\text{-}src}$: avian sarcomas c-Src truncation: colon cancer c-Src overexpression: mammary and pancreatic cancers, neuroblastomas, others
Fgr (c-*fgr*)	v-*fgr* from FeSV ($p70^{gag\text{-}fgr}$)	v-*fgr*: C-terminal truncated c-*fgr* and point mutations (increased kinase activity)	$p70^{gag\text{-}fgr}$: feline fibrosarcomas c-Fgr: acute myeloblastic leukaemias, chronic lymphocytic leukaemias, EBV-infected lymphomas; differentiation marker?
Yes (c-*yes*)	v-*yes* from ASV ($p90^{gag\text{-}yes}$)	v-*yes*: C-terminal truncated c-*yes* and point mutations (increased kinase activity) Over expression and/or increased kinase activity	$p90^{gag\text{-}yes}$: avian sarcomas c-Yes: colon carcinomas, malignant melanomas, other cancers
Lck		TCRβ-Lck (t(1; 7) T-cell receptor-β upstream of Lck causes overexpression) Overexpression GOF point mutations	TCRβ-Lck: T-cell acute lymphocytic leukaemias Lck: chronic lymphocytic leukaemias?
Abl (c-*abl*)	v-*abl* from Abelson MLV ($p160^{gag\text{-}abl}$) or from PI-FeSV	v-*abl*: N-terminal (ΔSH3) truncation of Abl Fusions: $p190^{Bcr\text{-}Abl}$, (t(9; 22) m-*bcr*); $p210^{Bcr\text{-}Abl}$, (t(9; 22) M-*bcr*); $p230^{Bcr\text{-}Abl}$, (t(9; 22) μ-*bcr*). M-, m- and μ-*bcr*: 3 breakpoint cluster regions in *BCR*. The chimaeric mRNA usually starts with exon a2 of *ABL*, it never includes exon 1a or 1b. Tel-Abl (t(12; 22) N-terminal (H-L-H region) of Tel fused with Abl exon 2a	$p160^{gag\text{-}abl}$: murine acute leukaemias $p190^{Bcr\text{-}Abl}$: ~50% of Ph$^+$ acute lymphocytic leukaemias, rarely chronic myelomonocytic leukaemias $p210^{Bcr\text{-}Abl}$: chronic myeloid leukaemias, ~30% of Ph$^+$ acute lymphocytic leukaemias $p230^{Bcr\text{-}Abl}$: some Ph$^+$ chronic neutrophilic leukaemias Tel-Abl: rare cases of acute lymphocytic leukaemias
Arg		Tel-Arg (t(1; 12))	Acute myeloid leukaemias (rare in M3 and M4Eo subtypes)
Jak1		Overexpression	Various leukaemias
Jak2		Tel-Jak2 (t(9; 12) H-L-H region (N-terminal) of Tel fused with kinase region (C-terminal) of Jak2)	T-cell childhood acute lymphocytic leukaemias, acute myeloid leukaemias, acute lymphocytic leukaemias, atypical chronic myeloid leukaemias
Jak3		Overexpression (increased kinase activity)	Various leukaemias and B-cell malignancies
Fak		Overexpression and/or altered tyrosine kinase activity	Modulation of adhesion, invasion and metastasis of diverse malignancies
Pyk2		Overexpression and/or altered tyrosine kinase activity	Modulation of adhesion, invasion and metastasis of diverse malignancies
Fes (c-*fes*)	v-*fps* from ASV; v-*fes* from FeSV ($p130$ and $p140^{gag\text{-}fps}$; $p135$ and $p140^{gag\text{-}fes}$)	The viral *gag* sequence essential for transforming activity. Viral *gag* fused to slightly truncated and/or point mutated *fps* or *fes*	$p130$ and $p140^{gag\text{-}fps}$: diverse avian sarcomas and myeloid leukaemias $p135$ and $p140^{gag\text{-}fes}$: feline sarcomas c-Fes: no implication in cancers
Brk		Overexpression (increased kinase activity)	Mammary carcinomas
Syk		Downregulation (recessive?)	Potential mammary carcinoma tumour suppressor; PTK-dependent?

*Abbreviations: AVE, avian erythroblastosis virus; FeSV, feline sarcoma virus; UR2 SV, UR2 sarcoma virus; RSV, Rous sarcoma virus; ASV, avian sarcoma virus; Abelson MLV, Abelson murine leukaemia virus; PI-FeSV, Parodi-Irgens feline sarcoma virus. Other abbreviations are defined in the text.
Limitations on space prevent the addition of references to the data presented in Table 1.

sequences[48]. The Bcr-Abl-tyrosine kinase domain is activated by formation of homo-oligomeric complexes mediated by the Bcr coiled-coil domain, allowing *trans* autophosphorylation[52]. The transforming effect of Bcr-Abl is mediated by numerous downstream signalling pathways normally activated by RPTKs, including the Ras-Raf-ERK, JAK-STAT and PI(3)K pathways[53]. The Bcr-Abl-activated pathways are very similar to those activated by Kit/SCFR[54]; the PI(3)K, and perhaps the JAK-STAT, pathways are essential for the mitogenic and anti-apoptotic transforming effects[55,56]. A requirement for Bcr-Abl kinase activity in transformation is demonstrated by the fact that the small-molecule c-Abl PTK inhibitor STI 571 (ref. 41) not only prevents cell growth of Bcr-Abl-transformed leukaemic cells, but also induces apoptosis in a manner dependent on Stat5-induced upregulation of the Bcl-2-like Bcl-x_L (ref. 57). Cytoplasmic retention of Bcr-Abl is in part kinase-dependent, so treatment of Bcr-Abl-transformed cells with STI 571 results in nuclear import. This observation can be used to enhance cell killing, by taking advantage of the pro-apoptotic effect of nuclear Abl. Treatment of STI 571-inhibited cells with leptomycin B, a nuclear export inhibitor, causes Bcr-Abl accumulation in the nuclear compartment; subsequent removal of STI 571 then results in re-activation of Bcr-Abl and apoptosis[48]. Although leptomycin B is too neurotoxic for use in treating patients, this rationale might have practical implications for combined use of STI 571 with a less toxic nuclear export inhibitor.

JAKs and STATs

The Janus PTKs (JAKs) have so far been implicated in a limited number of human leukaemias (Table 1), while some of their substrates, Stat3 and Stat5, are found in an activated phosphorylated state in several malignancies. The JAKs are cytoplasmic PTKs (Fig. 2) that mediate signalling primarily downstream of cytokine receptors (which lack catalytic domains), but also of RPTKs (see ref. 58 for a review). The seven known mammalian STATs are latent transcription factors with a central DNA-binding region and a C-terminal SH2 domain. In response to ligand, cytokine receptors become tyrosine-phosphorylated by the constitutively associated JAKs. Some of the phosphotyrosine residues subsequently bind STATs through their SH2 domains, which become phosphorylated by the oligomerized JAKs on a C-terminal tyrosine residue. This leads to STAT oligomerization through a reciprocal interaction between SH2 and phosphotyrosine[59]. Dimeric STATs are released from the receptors and translocate to the nucleus where they activate transcription. In

addition to activation by JAKs, STATs are also activated (either directly or indirectly) by RPTKs, G-protein-coupled receptors and cytoplasmic PTKs such as Src and Abl[60].

Ligand-induced STAT tyrosine phosphorylation is a transient and tightly controlled process lasting from minutes to hours[61]. Three types of inhibitors of STAT activation are known. The SHP-1 and SHP-2 PTPs directly dephosphorylate JAKs, and an unidentified nuclear PTP has been implicated in tyrosine dephosphorylation of Stat1, which might be important for inactivation and nuclear export of STATs[62]. The cytokine-inducible SH2-containing protein-1 (CIS-1)/suppressor of cytokine signalling-1, -2 and -3 (SOCS-1, -2 and -3)/JAK-binding protein (JBP)/STAT-induced STAT inhibitor-1 (SSI-1) family of proteins is transcriptionally upregulated by STATs, to inhibit STAT signalling. This occurs through SOCS-induced protein degradation, and, for SOCS-1 and -3, through binding of the N-terminal region to the activation loop of JAK kinase[63]. Finally, the protein inhibitors of activated STATs (PIAS) family of proteins inhibit phosphorylated STAT dimers from DNA binding and hence transcriptional activation. Inactivation of such negative regulators might be involved in tumorigenesis. A small deletion on chromosome 16 containing SOCS-1 has been identified in almost 50% of hepatocellular carcinomas.

Stat3 and Stat5 are overexpressed in some human malignancies. In head and neck cancer, Stat3 is persistently activated as a result of EGFR amplification, and has been shown to be required for v-Src-induced transformation. Moreover, expression of a constitutively active, dimeric Stat3 is transforming. Activation of Stat5 is implicated in human breast cancers[60]. A recurrent translocation involving the N-terminal dimerization domain of the ETS-like transcription factor TEL and Jak2, resulting in dimerization and constitutive activation of Jak2, has been reported in a few cases of T-cell acute lymphocytic leukaemia[64,65]. In a mouse model, Stat5 is essential for the Tel-Jak2-induced transformation[66]. Undoubtedly, the transforming properties of Stat3 and Stat5 depend on cross-talk with other signal-transduction pathways, in particular the Src and PI(3)K pathways. Thus, Jak1 and Jak2 bind and activate PI(3)K, activated Stat3 has been shown to activate Src and PI(3)K by direct binding through an interaction between phosphotyrosine and SH2, and Stat5 cooperates with PI(3)K in oncogenesis, for example, through induction of Bcl-x_L and inactivation of Bad[67–69].

PI(3)K and ribosomal S6 kinase/mTOR signalling

Numerous cytoplasmic protein-serine/threonine kinases, including Raf and ERK in the classical Ras-Raf-MEK-ERK pathway, are involved in cellular proliferation and have been linked to cancer. However, although mutationally activated or overexpressed Raf, Cot/Tpl2 and Mos protein-serine/threonine kinases can transform via the ERK/MAP kinase pathway, no frequently recurring mutations in protein kinases in the MAP kinase pathways have been identified in human malignancies. In contrast, mutations in several proteins in the PI(3)K/Akt and mammalian target of rapamycin (mTOR)/$p70^{S6K}$ signalling pathways, which regulate cell survival, growth and proliferation[70–74], are causally involved in a high percentage of common human malignancies, including mammary, prostate and colon carcinomas and malignant brain tumours.

PI(3)K

PI(3)Ks are a family of lipid kinases defined by their ability to phosphorylate the 3′-OH group of the inositol ring in inositol phospholipids. Class I PI(3)Ks are heterodimers made up of a catalytic subunit of relative molecular mass 110,000 (p110) and an adaptor/regulatory subunit. This class is further subdivided into the RPTK-activated subclass IA and the heterotrimeric G-protein-coupled receptor-activated subclass IB. The preferred substrate for class I PI(3)Ks in the intact cell is PtdIns(4,5)P_2. In addition, some of the class I and III PI(3)K members also exhibit protein-serine/threonine kinase activity, the functional role of which is still being explored.

There are currently three isoforms, α, β and δ, of the catalytic p110 subunit, and seven adaptor proteins generated by expression and alternative splicing of three genes, p85α, p85β and p55γ, in the class IA family of PI(3)Ks. Activation of class IA PI(3)Ks can occur by several means. RPTK activation leads to recruitment of PI(3)K, which binds through one or both SH2 domains in the adaptor to specific phosphotyrosine consensus motifs. This leads to allosteric activation of the catalytic subunit. In addition, Ras·GTP can bind directly to an N-terminal region in p110, leading to PI(3)K activation. Activation results in production of PtdIns(3,4,5)P_3 within seconds, and a slightly delayed production of PtdIns(3,4)P_2 through the actions of 5′-inositol lipid phosphatases. The effects of polyphosphoinositides in cells are mediated through the specific binding to at least two lipid-binding protein domains, the FYVE and pleckstrin-homology (PH) domains (see ref. 2 and http://smart.embl-heidelberg.de/ for protein modules in signalling). FYVE domains bind selectively to PtdIns(3)P, whereas a subgroup of PH domains, containing a highly basic motif, binds PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3. Proteins containing the latter domain are key mediators of class IA PI(3)K signalling. Phosphoinositide-binding PH domains are found in numerous proteins, including the protein-serine/threonine kinases, 3′-phosphoinositide-dependent kinase-1 (PDK-1) and Akt/protein kinase B (PKB), both central for the transforming effects of deregulated PI(3)K activity.

PDK-1 and PKB/Akt

PKB/Akt is the cellular homologue of the transforming viral oncogene v-Akt and bears significant homology to PKA and PKC[75]. The three mammalian isoforms, α, β and γ, all contain an N-terminal PH domain, a central kinase domain with an activation-loop Thr308 phosphorylation site, and a conserved, regulatory serine phosphorylation site, Ser473, near the C terminus. PDK-1 is a Thr308-Akt kinase, and only one mammalian isoform is known[76]. The C-terminal PH domain in PDK-1 binds phospholipids with around tenfold-higher affinity than the Akt PH domain, which probably explains the constitutive localization of PDK-1 at the plasma membrane. The following model for activation of Akt has been established (Fig. 3c): RPTK activation leads to production of PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 at the inner leaflet of the membrane. Akt interacts with these phospholipids, causing its translocation to the inner membrane, where PDK-1 is located. The interaction of the Akt PH domain with 3′-phosphoinositides is thought to impose conformational changes in Akt, exposing its two main phosphorylation sites. The PH domains might also mediate protein proximity between Akt and PDK-1 through homodimerization. PDK-1, believed to be constitutively active, subsequently phosphorylates Thr308 in Akt, which stabilizes the activation loop in an active conformation. This model is reminiscent of the general model for PTK activation (Fig. 3a–c). Phosphorylation of Thr308 is a prerequisite for kinase activation, but phosphorylation of the C-terminal hydrophobic residue is required as well for full activation of Akt kinase. The Akt Ser473 kinase ('PDK-2') remains to be identified (see ref. 76 for discussion). In a later phase, through unknown mechanisms, activated Akt is translocated to the nucleus where several of it substrates reside[77].

PDK-1 phosphorylates numerous other AGC kinase members in addition to Akt at the conserved activation-loop Ser or Thr residue, including several PKC isoforms, the serum- and glucocorticoid-induced kinases (SGKs), PKC-related kinase (PRK), $p70^{S6K}$ and p21-activated protein kinase (PAK; see Fig. 4). Results obtained using PDK-$1^{-/-}$ embryonic stem cells indicate that Akt, $p70^{S6K}$ and p90RSK are physiological PDK-1 substrates, while PDK-1 is not required for phosphorylation of PKA, mitogen- and stress-activated protein kinase-1 (MSK-1) and the AMP-activated protein kinase (AMPK; ref. 78). Additional evidence indicates that PKCζ and PKCδ, but not PRK, are also phosphorylated in the activation loop *in vivo* by PDK-1. The phosphorylation of the activation loop in $p70^{S6K}$ provided a long-sought link between PI(3)K and $p70^{S6K}$ activation.

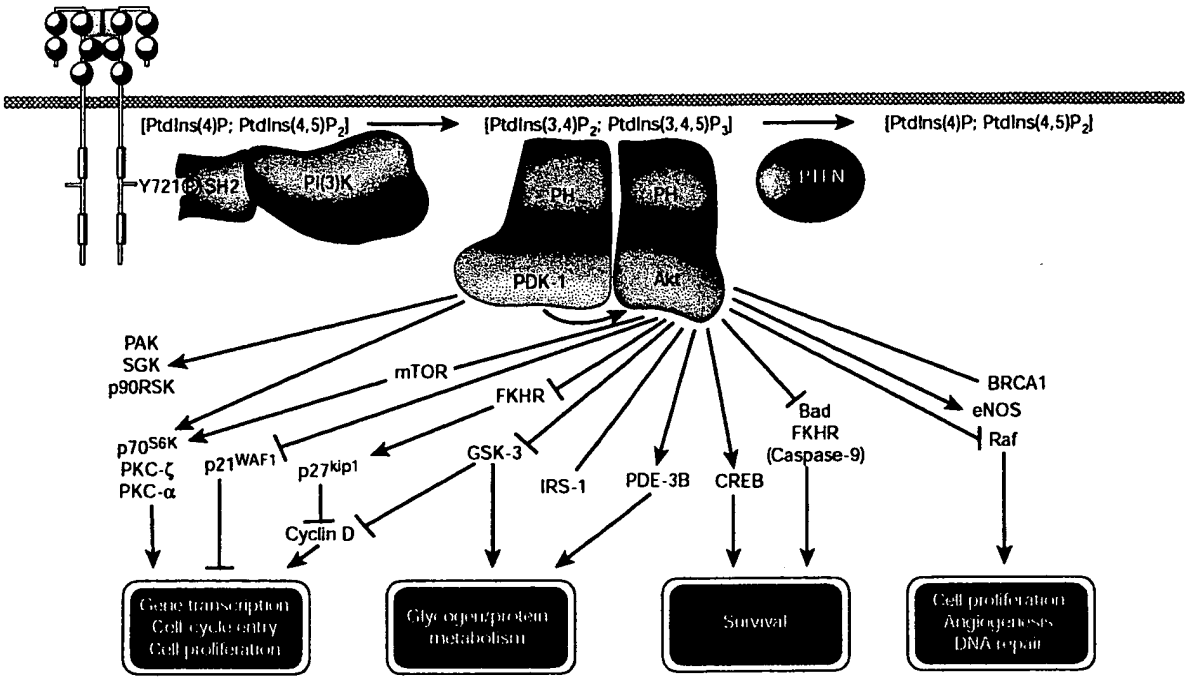

Figure 4 RPTK-induced PI(3)K signalling through PDK-1 and Akt. The figure illustrates signalling from the human Kit/SCFR, but the same general mechanisms apply to most RPTKs. See text for details.

At least 13 Akt substrates have been identified so far in mammalian cells, and they fall into two main classes: regulators of apoptosis on one hand and of cell growth, including protein synthesis and glycogen metabolism, and cell-cycle regulation on the other (Fig. 4). All identified substrates are phosphorylated within the same basic motif, R-X-R-X-X-S/T, which can also be phosphorylated by MAPKAPK-1 and $p70^{S6K}$. The Akt substrates involved in cell-death regulation include Forkhead transcription factors, the pro-apoptotic Bcl-2 family member Bad, and the cyclic AMP response element-binding protein (CREB). The anti-apoptotic effects of Akt-mediated phosphorylation of these have been extensively reviewed[79]. Glycogen synthase kinase-3 (GSK-3), phosphodiesterase-3B, mTOR, insulin receptor substrate-1 (IRS-1), the Forkhead member FKHR, the cyclin-dependent kinase inhibitor $p21^{CIP1/WAF1}$ and possibly Raf-1 are targets involved in mediating protein synthesis, glycogen metabolism and cell-cycle regulation. GSK-3 is inhibited by Akt phosphorylation. This abolishes phosphorylation of the cytoplasmic signalling molecule β-catenin, causing its stabilization and nuclear translocation. In the nucleus, it associates with T-cell factor/lymphocyte enhancer-binding factor-1 (TCF/LEF-1) to induce the transcription of several genes, including cyclin D1. This results in cell cycle progression through hyperphosphorylation and inactivation of Rb (see article in this issue by Evan and Vousden, pages 342–348). Cyclin D1 is also stabilized in this manner, owing to decreased phosphorylation at a GSK-3 site which promotes proteolytic turnover of cyclin D1 [80]. Phosphorylation of p21 by Akt causes its cytoplasmic retention, preventing it from exerting its anti-proliferative effects in the nucleus [81]. Phosphorylation of endothelial nitric oxide synthase (eNOS) and breast cancer susceptibility-1 (BRCA1) might regulate angiogenesis and DNA repair, among others (refs 70, 79, and see review in this issue by Hoeijmakers, pages 366–374).

The oncogenic role of deregulated class IA PI(3)Ks and Akt activity is probably accounted for by their ability to induce multiple simultaneous effects on both cell survival and cell cycle/cell growth. Aktβ is overexpressed in pancreatic and ovarian carcinomas, and the transforming effect of a constitutively active p110 found in a chicken tumour virus, p3k, is mediated through Akt[82]. Increased Akt kinase activity is correlated with p65 — an oncogenic mutant of p85α that induces constitutive PI(3)K activity — and amplification of p110 in ovarian cancer[83,84]. In addition, numerous human malignancies, including breast cancer, glioblastoma and germ cell tumours, are associated with inactivating mutations in the tumour-suppressor gene PTEN, leading to deregulated hyperactivity of Akt. PTEN is a 3′-phosphoinositide phosphatase, which dephosphorylates the 3′-OH position of the inositol ring in $PtdIns(3,4,5)P_3$ and $PtdIns(3,4)P_2$. Consequently, inactivating PTEN mutations lead to increased levels of 3′-phosphoinositides, causing enhanced Akt activity and cellular transformation. Part of the transforming effect of mutant PTEN and, as a consequence, deregulated Akt activity might occur through downregulation of the cyclin-dependent kinase inhibitor p27, a finding highly relevant to human prostate carcinoma[85]. Interestingly, as an apparent exception to these examples, disruption of p110γ, a class IB PI(3)K, is associated with colorectal cancers[86]. This was reportedly due to upregulation of Bcl-2, cyclin D and CDKs, but not of class IA PI(3)Ks, but the transforming mechanisms remain to be identified.

mTOR and ribosomal S6K

The phosphorylation of the ribosomal $p70^{S6K}$ by PDK-1 and of mammalian TOR (mTOR) by Akt provides mechanistic links between the two pathways, and recently it has become clear that some of the transforming, cell-growth- and cell-cycle-promoting effects of PI(3)K are mediated through the $mTOR/p70^{S6K}$ pathway. mTOR belongs to an evolutionarily conserved family of proteins, including TOR1, TOR2, MEC1, TEL1 and Rad3 in budding yeast, MEI-41 in the fruitfly *Drosophila*, and DNA-dependent protein kinase (DNA-PK), ATM, ATM-related (ATR), transformation/transcription domain-associated protein (TRAPP) and mTOR in mammals. mTOR is also called FK506-binding protein (FKBP)-rapamycin-associated protein (FRAP) in humans and rapamycin and FKBP12 target-1 (RAFT-1) in rats, based on the ability of FKBP–rapamycin complex to bind and inhibit mTOR. Two

mTOR-encoding genes have been identified, but only one has been studied.

Proteins in the mTOR family all have a C-terminal kinase domain with homology to the core kinase domain of PI(3)Ks and PtdIns(4)Ks, but only serine/threonine kinase activity has been demonstrated for these proteins[71]. Growth factors stimulate mTOR kinase activity, but the exact regulatory mechanisms are unknown. In response to insulin, Akt phosphorylates two sites in the C terminus of mTOR, but only one of these is a major site *in vivo*. However, this phosphorylation is not required for mTOR to phosphorylate two of its main substrates, eukaryotic initiation factor-4E (eIF-4E)-binding protein (4E-BP) and $p70^{S6K}$, which are involved in initiation of protein translation. Based on the use of the mTOR inhibitor rapamycin, it is clear that mTOR also regulates transcription of c-*myc* and is involved in activation of Stat3 by phosphorylation of Stat3 at Ser727 and of PKCα and PKCδ by phosphorylation at a conserved residue in their hydrophobic C-terminal motif. These effects might be involved in tumorigenesis[71,72]. The phosphorylation of 4E-BP causes dissociation from eIF-4E, enabling the latter to participate in cap-dependent initiation, including translation of mRNAs with a highly structured 5′-untranslated region, such as the transcripts encoding c-Myc and cyclin D1 involved in cell-cycle progression.

Two ribosomal S6 kinases, S6K1 and S6K2, are known, and they are key regulators of cell growth through control of the protein translational apparatus, in particular ribosomal proteins[73]. The shorter isoform of S6K1, $p70^{S6K}$, is largely cytoplasmic and its activation is blocked by rapamycin. $p70^{S6K}$ regulation is complex, and requires hierarchical phosphorylation. Activated $p70^{S6K}$ mediates the effects of mTOR on protein translation through its phosphorylation of the 40S ribosomal protein subunit S6, which drives translation of 5′-terminal oligopyrimidine-rich tract (5′-TOP) mRNAs. These mRNAs encode primarily ribosomal proteins and other protein components of the translational machinery[73]. However, $p70^{S6K}$ is also involved in cell-cycle regulation. Hence, $p70^{S6K}$ has been linked to PI(3)K-dependent proliferation through upregulation of cyclin D3 and resulting phosphorylation of Rb and p107, which leads to enhanced transcriptional activity of $E2F^{87,88}$. Transformation by PI(3)K and Akt, but not by numerous other oncoproteins, is dependent on phosphorylation and activation of $p70^{S6K}$ and phosphorylation of 4E-BP-1 by mTOR[89], thus establishing a clear link between PI(3)K, Akt, $p70^{S6K}$ and mTOR in oncogenesis.

Concluding remarks

Cancer is a multistep process, with accumulation of mutations in tumour-suppressor genes and dominant oncogenes. But the recent development of a series of relatively specific PTK inhibitors, and their ability to inhibit the proliferation of tumour cells expressing the target PTK *in vivo*, shows that inhibition of a deregulated, dominant oncogenic PTK is often enough to slow tumour progression. In consequence, much recent effort has gone into designing and identifying additional PTK inhibitors that are even more potent and specific. To facilitate drug testing there is a clear need for better animal models that precisely reflect the mutations in and pathogenesis of human malignancies. Most of the current refined animal models are based on conditional transgene expression and/or conditional gene knockouts. Not surprisingly, these models often give rise to a completely different tumour spectrum than that found in humans, and at best reflect a very late stage of the oncogenic process where chromosomal rearrangements have resulted in amplifications and deletions. Accordingly, only a few knockout models, in most cases involving deletions of tumour-suppressor genes which provide less attractive drug targets, provide realistic models (see for example, refs 85, 90). Most human cancers are caused initially by somatic point mutations, with only ~1% being due to germline mutations. In order to recapitulate the early events in human oncogenesis, including oncogenic lesions due to mutated PTKs, there is a need to develop mice with inducible/reversible site-directed point mutations.

Note added in proof. Initial results showing the efficacy and safety of STI571 for treating chronic myeloid leukaemia and Kit-positive gastrointestinal stromal tumours have just been published[91–93]. □

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Acknowledgements

We apologize to all colleagues, including those instrumental in generating the large amount of data compiled in Table 1, whose papers were not cited owing to space limitations. We thank M. R. Stratton and G. Pao for providing inspiration for Table 1, and J. Leverson for critical comments and suggestions. P.B.-J. is a Special Fellow of the Leukemia and Lymphoma Society of America and T.H. is a Frank and Else Schilling American Cancer Society Professor.